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Claims

What is claimed is:

1. A probe nucleic acid (PNA) comprising:

(a) a single-stranded sequence, 1/2 TBR, which is capable of forming, under hybridizing conditions, a hybrid, TBR, with a 1/2 TBR present in a target nucleic acid (TNA);

(b) a single stranded sequence, 1/2 BBR, which is capable of forming, under hybridizing conditions, a hybrid BBR, with about 0-10 1/2 BBR present in a booster nucleic acid (BNA); and

(c) an OSA, which is no attached support and/or indicator, or an attached support or other means of localization, including, but not limited to, attachment to beads, polymers, and surfaces, and/or indicators;

wherein said TBR is capable of binding with high affinity to a TBA, said TBA being a substance capable of discriminating between a paired TBR and a TBR having unpaired nucleotides, and further, wherein said BBR is capable of binding with high affinity to a BBA, said BBA being a substance capable of discriminating between a paired BBR and a BBR having unpaired nucleotides.

2. A booster nucleic acid (BNA) comprising:

(a) a 1/2 BBR which has a sequence which is complementary to a 1/2 BBR sequence in a PNA or another BNA and which is capable of forming, under hybridizing conditions, a hybrid, BBR, with the PNA;

(b) an OSA, which is no attached support or indicator or an attached support or other means of localization, including, but not limited to, attachment to beads, polymers, and surfaces, and/or indicators; and

(c) additional hybridization sites, 1/2 BBRs, for hybridization with additional BNAs;

wherein said BBR is capable of binding with high affinity to a BBA, said BBA being a substance capable of discriminating between a paired BBR and a BBR having unpaired nucleotides.

3. A Hairpin Nucleic Acid (HNA) comprising a single-stranded sequence, 1/2 BBR, which under hybridizing conditions is capable of forming a hairpin while at the same time binding to a BNA to form a BBR capable of binding a BBA, wherein said BBR is capable of binding with high affinity to a BBA, said BBA being a substance capable of discriminating between a perfect BBR and a BBR having unpaired nucleotides.

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4. The PNA of claim 1 wherein the TBR is comprised of one or more recognition sites for a nucleic acid binding protein, a DNA binding protein, a DNA-RNA hybrid binding protein or an RNA binding protein.

5. The PNA of claim 4 wherein the TBR is a nucleic acid binding protein recognition site present in the genome of a pathogen or is a binding site associated with a pathogenic condition in a vertebrate genome or is a nucleic acid binding protein recognition site present in the genome of an organism which contaminates a fermentation process.

6. The PNA of claim 4 wherein the TBR is the HIV-LTR or a portion thereof.

7. A method for detecting or localizing a specific TNA sequence, comprising the steps of:

(a) hybridizing said TNA with the PNA of claim 1;

(b) hybridizing said PNA with a BNA containing a 1/2 BBR whose sequence is complementary to a 1/2 BBR sequence in the PNA;

(c) adding the products of steps (a) and (b) containing a TBR and a BBR, to a surface, liquid or other medium containing a TBA;

(d) adding BBAs to the mixture in step (c) wherein said BBA comprises:

(i) a molecule or a portion of a molecule which is capable of selectively binding to a BBR;

(ii) a detectible indicator; and

(e) detecting signal produced by the indicator attached to the BBA.

8. The method of claim 7 wherein said indicator is a protein, including enzymes capable of catalyzing reactions leading to production of colored reaction products; a radionuclide; colored beads.

9. A method for detecting the presence in a sample of a specific Target Nucleic Acid, TNA, which comprises:

(a) contacting said sample with a Probe Nucleic Acid, PNA, which, upon hybridization with said TNA if present in said sample, forms a Target Binding Region, TBR, which is capable of binding a Target Binding Assembly, TBA; and

(b) contacting said sample, already in contact with said PNA, with a TBA capable of binding to any TBRs formed by the hybridization of said PNA and said TNA in the sample.

10. A method for detecting or localizing specific nucleic acid sequences with a high degree of sensitivity and specificity which comprises:

(a) adding PNAs containing a 1/2 BBR and a 1/2 TBR to a sample containing or suspected of containing TNAs containing 1/2 TBR sequences, to form a complex having target binding regions, TBRs, formed by the hybridization of complementary 1/2 TBRs present in the PNAs and TNAs respectively;

(b) binding the TBRs formed in step (a) to an immobilized TBA to form a TBA-TNA-PNA complex;

(c) adding Booster Nucleic Acids, BNAs, containing booster binding regions, 1/2 BBRs, to the complex formed in step (b) such that the 1/2 BBRs in the BNAs hybridize with the 1/2 BBR sequences present in the PNAs or to 1/2 BBRs present in BNAs already bound to the PNA, to form BBRs, such that TBA-TNA-PNA-$(BNA)_n$ complexes are formed;

(d) adding Hairpin Nucleic Acids, HNAs, containing 1/2 BBR sequences, to the complex formed in step (c) such that the 1/2 BBRs in the HNAs hybridize with any available 1/2 BBR sequences present in the BNAs of the complex of step (c), thereby capping the extension of the BNAs onto the TBA-TNA-PNA-$(BNA)_n$ complexes of step (c) to form TBA-TNA-PNA-$(BNA)_n$-HNA complexes;

(e) adding Booster Binding Assemblies, BBAs, linked to indicator moieties, to the TBA-TNA-PNA-$(BNA)_n$-HNA complexes formed in step (d) to form TBA-TNA-PNA-$(BNA\text{-}BBA)_n$-HNA complexes; and

(f) detecting the signals produced by the indicator moieties linked to the TBAs, PNAs, BNAs, BBAs or HNAs in the TBA-TNA-PNA-$(BNA\text{-}BBA)_n$-HNA complexes of step (e);

wherein the TNA comprises:

(i) one or more specific 1/2 TBR nucleic acid sequences, the presence or absence of which in a particular sample is to be confirmed;

the PNA comprises:

(i) a single-stranded sequence, 1/2 TBR, which is capable of forming, under hybridizing conditions, a hybrid, TBR, with a 1/2 TBR present in a target nucleic acid (TNA);

(ii) a single stranded sequence, 1/2 BBR, which is capable of forming, under hybridizing conditions, a hybrid BBR with a 1/2 BBR present in a booster nucleic acid (BNA); and

(iii) an OSA, which is no attached support and/or indicator, or an attached support or other means of localization, including, but not limited to, attachment to beads, polymers, and surfaces, and/or indicators;

the BNA comprises:

(i) a 1/2 BBR, as shown in Figure 1(IIb), which has a sequence which is complementary to a 1/2 BBR sequence in a PNA and which is capable of forming, under hybridizing conditions, a hybrid, BBR, with the PNA;

(ii) an OSA, which is no attached support or indictor or an attached support or other means of localization, including, but not limited to, attachment to beads, polymers, and surfaces, and/or indicators;

(iii) additional hybridization sites, 1/2 BBRs, for other BNAs; and

(iv) sequences, 1/2 BBRs, which can hybridize to BNAs already hybridized to the PNA;

the BBA comprises:

(i) a molecule or a portion of a molecule which is capable of selectively binding to a BBR; and

(ii) an OSA, which is no attached support and/or indicator, or an attached support or other means of localization, including, but not limited to, attachment to beads, polymers, and surfaces, and/or indicators;

and the TBA comprises:

(i) a molecule or a portion of a molecule which is capable of selectively binding to a TBR; and

(ii) no attached support and/or indicator, or an attached support or other means of localization, including, but not limited to, attachment to beads, polymers, and surfaces, and/or indicators.

11. In a solid phase hybridization method for detecting the presence of a target polynucleotide involving: immobilizing a target polynucleotide, if present in a test sample, directly

or via an intermediate capture structure, on a solid phase at a capture site; before, during or after said immobilization, attaching a detectable label to said target polynucleotide, if present; and detecting said label, if any, at said capture site; the improvement comprising:

(a) using a Target Binding Assembly, TBA, as the means for achieving immobilization of said target polynucleotide, wherein said TBA binds only to a unique hybrid formed between a specific Probe Nucleic Acid, PNA, and said target nucleic acid such that a perfect Target Binding Region, TBR, recognizable by said TBA is formed; and

(b) including in the PNA a single stranded sequence, 1/2 BBR, capable of binding a Booster Nucleic Acid, BNA, containing a single stranded complementary 1/2 BBR which, upon hybridization with the 1/2 BBR in the PNA, forms a BBR capable of binding labeled Booster Binding Assemblies, BBAs.

12. A target binding assembly, TBA, or a booster binding assembly, BBA, comprising at least one nucleic acid recognition unit, and optionally one or all of the sequences selected from the group consisting of a linker sequence, an assembly sequence, an asymmetry sequence, a nuclear localization signal sequence (NLS) and an OSA.

13. The TBA of claim 12 wherein the nucleic acid recognition unit is selected from the group consisting of an NF-kB binding unit, an SP1 binding unit, a TATA binding unit, a human papillomavirus E2 binding unit, an HPV LTR binding unit, an HIV LTR binding unit, and Tat.

14. The TBA of claim 13 wherein the nucleic acid recognition unit has the sequence selected from the group consisting of SEQ ID NO. 63, SEQ ID NO. 64, SEQ ID NO. 65, SEQ ID NO. 66, SEQ ID NO. 67, SEQ ID NO. 68, SEQ ID NO. 69, SEQ ID NO. 70, SEQ ID NO. 71, SEQ ID NO. 72, SEQ ID NO. 73, SEQ ID NO. 74, SEQ ID NO. 75, SEQ ID NO. 76, SEQ ID NO. 77, SEQ ID NO. 78, SEQ ID NO. 79, SEQ ID NO. 80, SEQ ID NO. 81, SEQ ID NO. 82, SEQ ID NO. 83, SEQ ID NO. 84, SEQ ID NO. 93, SEQ ID NO. 94, SEQ ID NO. 95, SEQ ID NO. 96, SEQ ID NO. 97, SEQ ID NO. 98, and SEQ ID NO. 118.

15. The TBA of claim 12 wherein the linker sequence is an oligopeptide which does not interfere with the nucleic acid recognition function of the nucleic acid recognition unit and which provides stability and control over the spacing of the nucleic acid recognition unit from the remainder of the TBA.

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16. The TBA of claim 15 wherein the linker sequence is an oligopeptide sequence from the interdomain primary sequence of a structural protein.

17. The TBA of claim 12 wherein the assembly sequence is an oligopeptide sequence which directs the folding and association of nucleic acid recognition units.

18. The TBA of claim 17 wherein the assembly sequence is derived from the bacteriophage lambda *cro* protein or the CI protein and is selected from the group consisting of SEQ ID NO. 104, SEQ ID NO. 105, SEQ ID NO. 106, SEQ ID NO. 107, and SEQ ID NO. 108.

19. The TBA of claim 12 wherein the asymmetry sequence directs the association of nucleic acid recognition and assembly sequences in a predetermined order.

20. The TBA of claim 19 wherein the asymmetry sequence is derived from insulin, gonadotropic hormone, FSH, HCG, LH, ACTH, or relaxin.

21. The TBA of claim 20 wherein the asymmetry sequence is selected from the group consisting of SEQ ID NO. 85, SEQ ID NO. 86, SEQ ID NO. 87, SEQ ID NO. 88, SEQ ID NO. 89, SEQ ID NO. 90, SEQ ID NO. 91, and SEQ ID NO. 92.

22. The TBA of claim 12 wherein the NLS is an oligopeptide which directs the migration and uptake of a protein or complex associated with said NLS into the nucleus of a cell.

23. The TBA of claim 22 wherein the NLS is selected from the group consisting of SEQ ID NO. 72 and SEQ ID NO. 103.

24. The TBA of claim 12 which is HIV Detect I-IV or HPV Detect I-IV.

25. The TBA of claim 12 having a sequence selected from the group consisting of SEQ ID NO. 109, SEQ ID NO. 110, SEQ ID NO. 111, SEQ ID NO. 112, SEQ ID NO. 113, SEQ ID NO. 114, SEQ ID NO. 115, and SEQ ID NO. 116.

26. A method of using the TBA of claim 12 to bind a particular nucleic acid sequence in a target nucleic acid sample which comprises:

(a) fragmenting the nucleic acid in the target nucleic acid sample;

(b) contacting, under hybridizing conditions, the fragmented nucleic acid with a probe nucleic acid complementary to the particular nucleic acid sequence of interest, wherein said probe nucleic acid, upon hybridization with said particular nucleic acid sequence of interest forms a target binding region to which said TBA specifically binds.

27. The method of claim 26 wherein said probe nucleic acid, in addition to sequences complementary to said particular nucleic acid sequence of interest, also has additional sequences to which a booster nucleic acid can bind to form a booster binding site to which a labeled booster binding assembly can bind to provide a signal showing and amplifying the binding of the probe nucleic acid to the target nucleic acid sequence of interest.

28. A method of using the TBA of claim 12 wherein said TBA is administered to patient in need of such treatment a therapeutically or prophylactically effective amount of said TBA, which comprises administering the TBA, either in the form of a purified protein complex or in the form of a recombinant vector which, upon entry into the patient is able to express the TBA, such that the TBA binds the particular nucleic acid sequence to achieve the desired prophylactic or therapeutic result.

29. The method of claim 28 wherein said TBA is selected from the group consisting of SEQ ID NO. 109, SEQ ID NO. 110, SEQ ID NO. 111, SEQ ID NO. 112, SEQ ID NO. 113, SEQ ID NO. 114, SEQ ID NO. 115, and SEQ ID NO. 116, and the patient is infected with HIV or HPV.

30. The method of claim 26 further comprising the step of:

(c) monitoring the shift in mobility of nucleic acids in the target nucleic acid sample as a function of the size such that binding of the TBA to a particular fragment in the sample modifies the mobility of the fragment.

31. A diagnostic or forensic test kit for the detection in a sample of nucleic acid having a specific sequence composition, which comprises:

(a) a first nucleic acid probe complementary to nucleic acid with specific sequence composition, the presence of which is to be ascertained in a test sample, wherein said first nucleic acid probe and said nucleic acid with specific sequence composition forming, upon hybridization, a binding site for a first nucleic acid binding protein, and wherein said first nucleic acid probe further comprises additional sequence complementary to a second nucleic acid probe;

(b) a first nucleic acid binding protein specific for the duplex formed by hybridization of said first nucleic acid probe and said nucleic acid with specific sequence composition;

(c) a second nucleic acid probe complementary to said additional sequence in said first nucleic acid probe, wherein, upon hybridization of said first and second nucleic acid probes, a binding site for a second nucleic acid binding protein is formed;

(d) a second nucleic acid binding protein which binds specifically to the duplex formed upon hybridization of said first nucleic acid probe and said second nucleic acid probe, wherein said second nucleic acid binding protein is labeled with a detectable label.

32. The diagnostic or forensic test kit of claim 31 wherein said first nucleic acid probe is complementary to the HIV LTR, such that upon hybridization of said first nucleic acid probe with an HIV LTR, a binding site is formed for NF-kB or a subunit thereof, SP1, TATA binding protein, HIV-Detect I, II, III, or IV, or HIV-Lock.

33. The diagnostic or forensic test kit of claim 32 wherein said first nucleic acid binding protein is NF-kB or a subunit thereof, SP1, TATA binding protein, HIV-Detect I, II, III, or IV, or HIV-Lock.

34. The diagnostic or forensic test kit of claim 33 wherein said first nucleic acid probe, in addition to being complementary to the HIV LTR, comprises a sequence encoding the bacteriophage lambda left or right operator and said second nucleic acid probe comprises sequences complementary to said bacteriophage lambda left or right operator sequences in said first nucleic acid probe, such that upon hybridization of said first and second nucleic acid probes, a binding site for the bacteriophage lambda CI repressor protein, the bacteriophage lambda *cro* protein or a derivative or homology thereof, is formed.

35. The diagnostic or forensic test kit of claim 34 wherein said second nucleic acid binding protein is the bacteriophage lambda CI repressor protein, the bacteriophage lambda *cro* protein or a derivative or homology thereof.

36. A composition comprising HIV-Lock or a recombinant vector encoding HIV-Lock and a pharmaceutically acceptable carrier.

37. A method of differentially binding a nucleic acid binding protein to a nucleic acid sequence correlated with a pathogenic condition which comprises:

(a) selecting a particular configuration of nucleic acid binding protein sequences present in the nucleic acid sequence correlated with a pathogenic condition as a target sequence for designing a probe nucleic acid which will hybridize to that particular configuration of nucleic acid sequences if present in a test sample, and further, ensuring that a binding site for an available nucleic acid binding protein is formed upon hybridization of said probe nucleic acid and said particular configuration of nucleic acid sequences chosen as a target;

(b) selecting a nucleic acid binding protein which specifically binds to the selected particular configuration of nucleic acid binding protein sequences correlated with a pathogenic condition, but which does not bind to sequences not correlated with said pathogenic condition;

(c) hybridizing said probe nucleic acid with a test sample suspected of containing said particular configuration of nucleic acid binding protein sequences present in nucleic acid sequences correlated with a pathogenic condition;

(d) contacting said nucleic acid binding protein with any hybrids formed in step (b); and

(e) detecting any binding of said nucleic acid binding protein with said hybrids.

38. The method of claim 37 wherein said particular configuration of nucleic acid binding protein sequences is chosen from a necessary step or control point in the development of a pathogenic condition.

39. The method of claim 9 wherein said method is carried out in an automated fashion.

40. The method of claim 39 wherein the method is carried out in the Abbott Laboratories IMx machine.

41. The method of claim 9 carried out in a microtiter plate.

42. A method of amplifying the signal obtained through binding the PNA of claim 1 to a TNA which comprises binding BNAs to the PNA-TNA hybrid and binding labeled BBAs to the BNAs.

43. A method of assembling a nucleic acid binding complex which comprises using asymmetry sequences to direct the association or non-association of components of the nucleic acid binding complex.

44. A method of assembling a nucleic acid binding complex which comprises using assembly sequences derived from bacteriophage lambda *cro* or CI to assemble associated components of the nucleic acid binding complex.

45. A method of using assembly, asymmetry, or piloting sequences to assemble a multimeric protein complex which comprises linking subunits to be incorporated into the multimeric protein complex to said assembly, asymmetry, or piloting sequence, and recovering said multimeric complex.

46. A composition comprising SEQ ID NO. 105, SEQ ID NO. 106, or SEQ ID NO. 108.

47. A nucleic acid encoding the TBA or BBA of claim 12.

48. The TBA of claim 12 or a nucleic acid encoding said TBA, wherein the amino acid sequence of said TBA is selected from the group consisting of set A, set B and set C, wherein said sets are comprised as follows:

Set	Link Sequences from Groups
A	I + II + III
B	IV + V + III
C	IV + III

wherein groups I-V consist of sequences selected from:

Group	Selected from Sequences
I	Any of SEQ ID NOS. 85-92
II	Met Ser, linked to any of SEQ ID NOS. 104-106, each of which is linked to SEQ ID NO. 99

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III	SEQ ID NO. 100 linked to any of SEQ ID NOS. 75-84 or 94-98; SEQ ID NO. 101 linked to either SEQ ID NO. 74 of SEQ ID NO. 93; or SEQ ID NO. 102 linked to SEQ ID NO. 74 or SEQ ID NO. 93; or any of SEQ ID NOS. 72, 103, 73, or 63-71
IV	Any of SEQ ID NOS. 104-108
V	SEQ ID NO. 99.

49. A method of assembling multimeric TBAs *in vivo* or *in situ* which comprises introducing component TBAs into a cell utilizing a covalently or non-covalently attached protein or bi-layer vesicle or by introducing nucleic acids encoding component TBAs into a cell, said component TBAs each comprising a DNA recognition unit, assembly sequences, asymmetry sequences, nuclear localization signal sequences, and optional linker sequences, such that upon proximal binding via the DNA recognition unit of each component TBA to nucleic acid sequences encountered in the nucleus or elsewhere in the cell, component expressed TBAs assemble via said assembly and asymmetry sequences into multimeric TBAs.

50. A method for identifying nucleic acid binding molecules for preparation of a target binding assembly or a booster binding assembly comprising:

a. Obtaining a sample containing the target nucleic acid;

b. Fragmenting the sample so as to expose the nucleic acids and to reduce the size complexity of the nucleic acids contained in the sample;

c. Contacting a first aliquot of the fragmented nucleic acids with a control buffer medium and contacting a second aliquot of the fragmented nucleic acids with the control buffer medium containing a known profile of nucleic acid binding molecules;

d. Analyzing the two aliquots to identify fragments which have altered behavior in the aliquot contacted with the target binding molecules as opposed to the aliquot contacted with the control buffer medium;

e. Identifying and isolating fragments which do exhibit altered behavior when contacted with the nucleic acid binding molecule and either sequencing the nucleic acid fragment to determine whether known nucleic acid binding molecule motifs are present, or directly identifying the nucleic acid binding molecule bound to the nucleic acid; and

f. synthesizing TBAs comprising the nucleic acid binding molecules which produced the altered behavior using assembly, asymmetry nuclear localization and, optionally, linker sequences.

51. A method for identifying specific nucleic acid sequences in a sample comprising:

a. Fragmenting the nucleic acids in said sample to expose the nucleic acids and reduce the size complexity of the nucleic acids;

b. Contacting a TBA with the sample, said TBA comprising two or more nucleic acid binding components each of which has a relatively weak binding for its nucleic acid recognition unit within the TBR but which in combination provides strong binding for the complete TBR; and

c. Eliminating any "cross-talk" produced by binding of the TBA to cousin nucleic acids that contain individual recognition units, which comprises contacting the sample with excess nucleic acid binding components with relatively strong binding affinity for cousin nucleic acids that contain the individual recognition units but relatively weak binding relative to the TBA's affinity for binding to the complete TBR having said two or more nucleic acid binding components.